REMARKS

In the specification, the paragraph beginning at page 1, line 1 has been amended to correct the priority claim.

Claims 24-26, 29, 30, 33, 35, 36, 38, 39, 41, and 42, to the extent that they are drawn to polynucleotides encoding amino acids 299-396 of Figure 2, remain pending in the application. Claims 1-23, 27, 28, 31, 32, 34, 37, 40, and 43-49 have been cancelled. Claims 24-26, 29, 30, 33, 35, 36, 41, and 42 have been amended to more clearly define the claimed subject matter. Applicants reserve the right to pursue the subject matter of all cancelled claims in one or more divisional applications.

OBJECTION TO PRIORITY INFORMATION

The Examiner has requested correction of the paragraph containing continuing data. Applicants have amended the specification to provide the correct history of the application. To clarify, U.S. Serial No. 07/721,847 is a continuation-in-part of three separate applications: 07/493,272; 07/378,537; and 07/655,579. 07/655,579 is a divisional of 07/179,100. Applicants believe that the amended paragraph accurately reflects the proper continuing data.

REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 33, 36, 39, and 42 have been rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. The Examiner contends that the definition of "stringent hybridization conditions" in claim 33 is unclear and suggests that Applicants incorporate the actual conditions into the claim.

Applicants traverse. The claim term "stringent hybridization conditions" is clearly defined in the specification at page 23, lines 25-27 and in the enclosed copy of Maniatis et al., Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982). In addition, Applicants submit copies of U.S. Patent Nos. 6,733,965; 6,734,003; 6,734,009; 6,734,293; 6,743,907; and 6,746,861, all of which issued with claims reciting "stringent conditions" in the absence of specific conditions in the claims. Applicants submit that it is proper practice for the claims to refer to hybridization conditions defined in the specification and request that the Examiner withdraw the rejection of claim 33 and claims 36, 39, and 42, which depend from claim 33.

Claim 42 has been rejected under 35 U.S.C. § 112, second paragraph, as allegedly indefinite. The Examiner indicates that "BMP-2" should be replaced with "bone morphogenetic protein-2," to more clearly identify the claimed subject matter. Applicants have made the suggested claim amendment and request that the Examiner withdraw this rejection.

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claims 24, 35, and 38 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking written support in the specification. The Examiner objects to the language "naturally occurring allelic variants" and contends that the specification does not provide any definition of the term "allele," nor does it describe which nucleotides may vary in these allelic variants. Applicants traverse.

The language "allelic variant" is clearly defined in the specification. Specifically, on page 7, lines 4-18, the specification describes:

> "factors into which modifications are naturally provided (allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) . . . These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics may possess bone growth factor biological properties in common. Thus, they may be employed as biologically active substitutes."

On page 8, lines 10-16, the specification describes sequences that "differ in codon sequences due to degeneracies of the genetic code or allelic variations (naturally occurring base changes in the species' population which may or may not results in an amino acid change)." Applicants submit that with this definition of "allelic variant," one skilled in the art would readily understand how the sequence disclosed in Figure 2 is representative of other allelic variants.

In addition, Applicants have provided the necessary information for one skilled in the art to identify and isolate nucleotides encoding BMP-2 from natural sources, which would provide the claimed naturally occurring allelic variants. See, e.g., Examples IV and V, which describe the isolation of bovine and human sequences encoding BMP-2. Accordingly, Applicants submit that the language "naturally occurring allelic variants" has written description support in the specification.

Finally, Applicants submit that the term "allelic variant" is well understood by those of skill in the art. The term is commonly allowed by the U.S.P.T.O. in claims recited DNA or amino acid sequences. For example, Applicants direct the Examiner's

attention to U.S. Patent Nos. 5,846,770; 5,849,880; 5,932,216; 5,948,639; and 5,586,388, which all claim allelic variants. U.S. Patent Nos. 5,586,388 and 5,948,639 do not contain a definition of this term in the specification. U.S. Patent Nos. 5,846,770; 5,849,880; and 5,932,216 (all assigned to Genetics Institute) have the same definition of allelic variants used in this application: sequences that "differ in codon sequences due to degeneracies of the genetic code or allelic variations (naturally occurring base changes in the species' population which may or may not results in an amino acid change)." Accordingly, Applicants submit that the "naturally occurring allelic variants" claim language is supported by the specification and allowable. Applicants request that this rejection of claims 33, 36, 39, and 42 be withdrawn.

Claims 33, 36, 39, and 42 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. The Examiner contends that sequences capable of hybridizing to the sense strand of the sequence of Figure 2 will not encode a protein with bone morphogenetic activity. Applicants have amended claim 33, which now recites a DNA molecule that hybridizes to a sequence complementary to the sequence of Figure 2. The claimed DNA molecule will contain a sequence similar to that of the sense strand of Figure 2 and will encode a BMP-2 protein. Applicants request that the Examiner withdraw this rejection of claim 33, and dependent claims 36, 39, and 42.

REJECTIONS UNDER 35 U.S.C. § 101

Claims 24-26, 29, 30, 33, and 42 have been rejected under 35 U.S.C. § 101 as directed to DNA sequences, which are not patentable subject matter. As suggested by the Examiner, Applicants have amended these claims to recite "isolated polynucleotide" instead of "DNA sequence." In light of this amendment, Applicants request that the claim rejections under 35 U.S.C. § 101 be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully requests reconsideration and reexamination of this application and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to Deposit Account No. 06-0916.

Respectfully submitted,

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Dated: September 9, 2004

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Attachments:

- Maniatis et al., Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387-389.
- U.S. Patent No. 6,733,965
- U.S. Patent No. 6,734,003
- U.S. Patent No. 6,734,009
- U.S. Patent No. 6,734,293
- U.S. Patent No. 6,743,907
- U.S. Patent No. 6,746,861
- U.S. Patent No. 5,846,770
- U.S. Patent No. 5,849,880
- U.S. Patent No. 5,932,216
- U.S. Patent No. 5,948,639
- U.S. Patent No. 5,586,388

APPLICANT

2

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

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UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

E.F. Fritsch
GENETICS INSTITUTE

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Cold Spring Harbor Laboratory Press
1989

concentrations of protein are believed to interfere with the annealing of the probe to its target. This quenching of the hybridization signal is particularly noticeable when oligonucleotides or probes less than 100 nucleotides in length are used.

7. In the presence of 10% dextran sulfate or 10% polyethylene glycol, the rate of hybridization is accelerated approximately tenfold (Wahl et al. 1979; Renz and Kurz 1984; Amasino 1986) because nucleic acids are excluded from the volume of the solution occupied by the polymer and their effective concentration is therefore increased. Although dextran sulfate and polyethylene glycol are useful in circumstances where the rate of hybridization is the limiting factor in detecting rare sequences (e.g., northern or genomic Southern blots), they are of no benefit when screening bacterial colonies or bacterial plaques. In addition, they can sometimes lead to high backgrounds, and hybridization solutions containing them are always difficult to handle because of their viscosity. We therefore recommend that dextran sulfate and polyethylene glycol not be used unless the rate of hybridization is very slow, the filter contains very small amounts of DNA, or the amount of radiolabeled probe is limiting.

8. To maximize the rate of annealing of the probe with its target, hybridizations are usually carried out in solutions of high ionic strength (6 × SSC or 6 × SSPE) at a temperature that is 20–25°C below the melting temperature (T_m). Both solutions work equally well when hybridization is carried out in aqueous solvents. However, when formamide is included in the hybridization buffer, 6 × SSPE is preferred because of its greater buffering power.

9. In general, the washing conditions should be as stringent as possible (i.e., a combination of temperature and salt concentration should be chosen that is approximately 12–20°C below the calculated T_m of the hybrid under study). The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the probe of interest and then washed under conditions of different stringencies.

10. To minimize background problems, it is best to hybridize for the shortest possible time using the minimum amount of probe. For Southern hybridization of mammalian genomic DNA where each lane of the gel contains 10 μg of DNA, 10–20 ng/ml radiolabeled probe (sp. act. = 10^9 cpm/μg or greater) should be used and hybridization should be carried out for 12–16 hours at 68°C in aqueous solution or for 24 hours at 42°C in 50% formamide. For Southern hybridization of fragments of cloned DNA where each band of the restriction digest contains 10 ng of DNA or more, much less probe is required. Typically, hybridization is carried out for 6–8 hours using 1–2 ng/ml radiolabeled probe (sp. act. = 10^9 cpm/μg or greater).

11. *Useful facts*:

    a. The T_m of the hybrid formed between the probe and its target may be estimated from the following equation (Bolton and McCarthy 1962):

$$T_m = 81.5°C + 16.6(\log_{10}[Na^+]) + 0.41(\text{fraction G + C}) - 0.63(\%\,\text{formamide}) - (600/l)$$

where l = the length of the hybrid in base pairs.

This equation is valid for:

- Concentrations of Na^+ in the range of 0.01 M to 0.4 M. It predicts T_m less accurately in solutions of higher $[Na^+]$.
- DNAs whose G + C content is in the range of 30% to 75%. Note that the depression of T_m in solutions containing formamide is greater for poly(dA:dT) (0.75°C/1% formamide) and less for DNAs rich in poly(dG:dC) (0.50°C/1% formamide) (Casey and Davidson 1977).

The equation applies to the "reversible" T_m that is defined by optical measurement of hyperchromicity at OD_{257}. The "irreversible" T_m, which is more important for autoradiographic detection of DNA hybrids, is usually 7–10°C higher than that predicted by the equation.

Similar equations have been derived for:

i. RNA probes hybridizing to immobilized RNA (Bodkin and Knudson 1985)

$$T_m = 79.8°C + 18.5(\log_{10}[Na^+]) + 0.58(\text{fraction G + C}) + 11.8(\text{fraction G + C})^2 - 0.35(\%\,\text{formamide}) - (820/l)$$

ii. DNA:RNA hybrids (Casey and Davidson 1977)

$$T_m = 79.8°C + 18.5(\log_{10}[Na^+]) + 0.58(\text{fraction G + C}) + 11.8(\text{fraction G + C})^2 - 0.50(\%\,\text{formamide}) - (820/l)$$

Comparison of these equations shows that the relative stability of nucleic acid hybrids decreases in the following order: RNA:RNA (most stable), RNA:DNA (less stable), and DNA:DNA (least stable). In aqueous solutions, the T_m of a DNA:DNA hybrid is approximately 10°C lower than that of the equivalent RNA:RNA hybrid. In 80% formamide, the T_m of an RNA:DNA hybrid is approximately 10°C higher than that of the equivalent DNA:DNA hybrid.

b. The T_m of a double-stranded DNA decreases by 1–1.5°C with every 1% decrease in homology (Bonner et al. 1973).

The above equations apply only to hybrids greater than 100 nucleotides in length. The behavior of oligonucleotide probes is described in detail in Chapter 11.

For a general discussion of hybridization of nucleic acids bound to solid supports, see Meinkoth and Wahl (1984).